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19 ABSTRACT (Continue on reverse if necessary and identify by block number) The <u>lux</u> genes of <u>Vibrio fischeri</u> encode the ability of this marine bacterium to produce light. <u>V. fischeri</u> occurs at high density in specialized light-emitting organs of certain marine fish, where the light produced is used by the fish. <u>V. fischeri</u> is also found in seawater, where it exists as a member of the bacterioplankton. In the planktonic habitat light-production is not useful and in fact <u>V. fischeri</u> possesses a genetic control mechanism which enables light production when the bacteria exist in the symbiotic state but does not allow synthesis of the light-emitting system when <u>V. fischeri</u> is in the planktonic habitat. This regulatory phenomenon is termed auto-induction, and the aim of this research is to fully elucidate the mechanism of auto-induction. Specific objectives of this research effort include a structure/function analysis of the sensory receptor; the LuxR protein, purification of the LuxR protein and development of a defined in vitro assay for studying <u>lux</u> gene transcription.			
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Date: 1 June 1989

PROGRESS REPORT ON CONTRACT N00019-88-K-0570 R & T Code 441d019

PRINCIPAL INVESTIGATOR: Everett P. Greenberg

CONTRACTOR: University of Iowa

CONTRACT TITLE: Regulation of *lux* Genes in *Vibrio fischeri*: Control of a Symbiosis-related Gene Expression System in a Marine Bacterium

START DATE: 15 August 1988

RESEARCH OBJECTIVE: To elucidate the mechanism of autoinduction of the *Vibrio fischeri lux* genes and to understand some of the physical factors which affect autoinduction. Specific objectives include development of an in vitro assay for autoinducer controlled transcriptional activation of *lux* genes, determination of the nature of the autoinducer interaction with its receptor, and the nature of the receptor interaction with DNA.

PROGRESS (Year 1): In the initial year of this ONR project, we have studied mutations in *luxR*; the gene encoding the transcriptional activator of *V. fischeri* luminescence. We have also made considerable progress towards purification of the *luxR* product for studies of the activity of this protein.

A number of *luxR* point mutations have been obtained. One class encodes LuxR proteins which do not activate transcription of the other *lux* genes. These mutants were isolated in the following manner: Hydroxylamine mutagenesis of pHK724 (a plasmid containing *luxR* under control of the *tac* promoter) was performed in vitro. The pool of mutagenized plasmids was used to transform *E. coli* cells containing pHK555 a plasmid compatible with pHK724 which possesses functional copies of all the *lux* genes except *luxR*. Transformants incapable of light production were considered *luxR* mutants. Sixteen such mutants were obtained and the plasmids derived from pHK724 were transferred to *E. coli* JM109 without pHK555. Each of these strains was screened by Western immunoblot procedures using anti-LuxR rabbit antiserum. Of the 17 strains, ten produced proteins of the appropriate molecular weight. Apparently the plasmids in these strains carried *luxR* missense mutations. The mutations in the ten *luxR* mutant genes encoding detectable protein have been sequenced. In nine, the mutations give rise to a single amino acid residue replacement. These mutations cluster in two domains of the 250 amino acid residue LuxR protein. Three are in a region between residue 94 and residue 127 and six are in a region between residues 184 and 230. Based on homologies with a group of other DNA binding protein, Henikoff, Wallace and Brown (Methods in Enzymology, In press), suggested the LuxR DNA-binding domain was around residues 200-220. Thus, the mutant analysis suggests the region between residue



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184 and 202 is in the DNA-binding region. One would deduce then that the region defined by the mutations changing residues between 94 and 127 defines the area important to the autoinducer interaction. In support of this conclusion, the phenotype of the mutant encoding a protein with a substitution at residue 127 is reversible by exogenous addition of autoinducer.

The second aspect of the project that we have initiated involves purification of LuxR. Previously, we overexpressed *luxR* in *E. coli* and purified the overexpressed protein product. However, the overexpressed protein was synthesized in the form of inactive inclusion bodies. We have used antibodies raised against the pure but inactive LuxR protein as an analytical tool to detect soluble LuxR in cell extracts. In fact when the inclusion bodies are removed from extracts of *E. coli* a significant amount of soluble LuxR remains as detected by Western immunoblotting. This protein cannot be visualized by commassie blue staining of SDS-gels of these extracts. We have partially purified the soluble LuxR from these extracts. The approach may be of general utility in cases where inclusion bodies form upon overexpression of a foreign protein in *E. coli*.

WORK PLAN (Year 2): The mutations described define two regions in the terminal two-thirds of the LuxR protein that are important to activity. Thus, I now plan on deletion analysis in which parts of the 5'-third of *luxR* will be excised in order to produce shorter proteins. I anticipate that analysis of strains carrying these mutations will lead to some understanding of the function of the N-terminal region of LuxR.

We also intend to completely purify the LuxR protein and initiate our analysis of its activity in vitro.

INVENTIONS (Year 1): No inventions.

PUBLICATIONS AND REPORTS (Year 1):

1. A manuscript describing the analysis of *luxR* point mutations is in preparation.

TRAINING ACTIVITIES: One Postdoctoral Fellow (Dr. James Slock) and one graduate student (Ms. Dana Kolibachuk) have been working on this project since August, 1988. A second graduate student (Mr. Sang Ho Choi) joined the lab to work on this project in March, 1989. Stipends for each of these individuals are provided by sources other than this contract. Dr. Slock left in July to assume a faculty position at King's College in Pennsylvania. Dr. Kendall Gray joined the group in August, 1989 as a Postdoctoral Associate. The demographic data regarding these students are

Women or minorities - 1
Non-citizens - 1 (citizen of Korea)

AWARDS/FELLOWSHIPS: Elected fellow of AAAS, Dr. Greenberg, January, 1989. Sea Grant Postdoctoral Scholar Award to Dr. James Slock, August, 1988. Sea Grant Postdoctoral Scholar Award (Continuation) to Ms. Dana Kolibachuk.

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